Electronic Supplementary Information to the Publication

Assessment of the rules related to gaining activity against Gram-negative bacteria

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1.) Biological Results

1.1 Cloning, Expression and Purification of *Plasmodium falciparum* IspE

Table S1 Primers used for amplification of the DNA fragment coding for the IspE of *P. falciparum.*

Primer	Primer sequence (5' → 3')
*Pf*IspE-NcoI-cHis6-fw	ATAATAATACCATGGGACATCATCACCACCATCATGGCAGCAATGTGGAAAAGAACAACG
*Pf*IspE-cHis6-HindIII-bw	TGTTGTTGTAAGCTTACTTGAACTCATGCGCTAGCTTGATCGGGTCG

CCATGGGAAATGTGGAAAAGAACAACGTGGTTAACACTAATAAGGAGATCGAGAAACTGCTCTTAGATGTATTGGACAACCGTAATAACTGGTACGATTC
AAAGTACTTCTCTCCGGCGAAAATTAATCTGTTTCTGCGCTTGAAGGAAAAGAAAGAGACGTACAATGAAGTATCAACCCTCATGCATTCACTGAATTTGG
GCGATGACATCTTCATTCGCGCCTTGAAGAAAGAAGATCAGAATAAGCTGCGCCATTTCTTACACCCGTGCGAGTCTGGCGACTTCTTGACAATTGTACGT
ATGGAAGATAAGAACCGCGATAAGGAAACGTTAAAAGAAGACTGCAAGATCGATGTGATCAATAAGAGTGATAAGGACTTGTTTAAACACATGAAAGAG
GACATCATTATCCAAGAACACGAGAAATTACCGTACGAGTACAATGACTACCCGATCAATAACGATAATATCATCATTAAGGTGTTAAAGCGCTATCGTGA
GGAATTCAATATCAGTGATGACATCCGCTTTCTGATCCACGTGAATAAGCGCATCCCGATCTTTAGTGGCGTGGGTGGTGGGAGCTCTAATGGTGCGACC
GTTTTCTATTTCTTAGAGAACATCTTTTACAAGTATTTTAAAGGCGACAATATCAAAGCGAACGAGTTCTTAAAGACTATCGGCAGCGACATCTCCTTTTTCA
GTAGCTCGGGCTTTGCGTATTGCACGGATAAGGGCAATAACGTGACGGATTTAAAAAACATCGAAGCGAATATCAAGGACAAAGATATCTATTTATTCAA
GATTGATGAGGGCTTATCTTCGAAACTAGTATACAAAAATGTCGACTACAAGCGCATTATCCAATATAACCCGGTGAATCTGCTTAAGTGCTTAATTAACAC
ATCAAATGATGACATCATCAAACAGATCGAGGAAAAGGAGAAGAAATTTGCCAACACTTTCATTTCGCTGGATAATCGCGATAACCTGCAAAATGTGTTC
GTGAATGACCTCGAGCACTCAGCGTTTTACTTAATCAAAAAGCTGCAGGATTTAAAGGAATATCTGCGCAGTCAAAACATGTTTGACGTCGTGTCCATGAG
CGGCAGTGGCTCTTCGCTGTTTGCCTTGTCCAACAAGAAAACGCAAACTCATGAGATCTCTTCATCGTTTCAAAACGAACGCATTAAGAAACTCATCAGCG
ACATCAAGATTAAATTCAACATGAATGTACGCGTCTATCTGTGCGATGCGCTCCGTAAAGGCCTTGATGTGTGGTACGACCCGATCAAGCTAGCGCATGA
GTTCAAGGGCAGC**CATCATCACCACCATCAT**TAAGCTT

Fig. S1 The nucleotide sequence of the DNA fragment coding for IspE from *P. falciparum*. The restriction sites NcoI and HindIII are underlined. The sequence coding for the His_6-Tag is shown in bold.

1.2. Summary of Enzymatic Results

Table S2 Inhibitory activities against IspE enzymes and auxiliary enzymes PK/LDH.

Compound	*Ec*IspE IC_{50} (µM)	*Pf*IspE IC_{50} (µM)	PK/LDH IC_{50} (µM)
1	1 ± 0	>500	>500
2	91 ± 21	57 ± 12	65 ± 15
3	68 ± 13	35 ± 6	56 ± 11
8	200 ± 62	33 ± 11	200 ± 9
9	>500	61 ± 7	>500
10	>500	37 ± 2	159 ± 28
11	>500	196 ± 44	>500
12	>500	113 ± 40	>500
13	200 ± 35	>500	>500
14	>500	>500	n.d.
15	>500	>500	n.d.
16	>500	>500	n.d.
17	>500	>500	n.d.
21	>500	>500	n.d.
22	>500	>500	n.d.
23	>500	>500	n.d.
24	>500	>500	n.d.
25	>500	>500	n.d.
26	>500	>500	n.d.

* The results are from at least two independent determinations. Only where *Pf*IspE or *Ec*IspE activity was measured as >500, no replicate was determined. PK/LDH: pyruvate kinase and lactate dehydrogenase and n.d.: not determined.

1.3. Summary of Cellular Results

Table S3 Inhibitory activities against a panel of *Escherichia coli* strains, *Staphylococcus aureus*, *Plasmodium falciparum* and the human hepatoma cell line HepG2, from at least two independent determinations.

	Percentage Inhibition @ given concentration							**IC_{50}**
Compound	***E. coli***					***S. aureus*** **Newman**	**HepG2**	***Pf*NF54**
	K12	**Δ*tolC***	**Δ*acrB***	**D22**	**(DE3) omp8**			
1	6 ± 1 (@100 µM) 1 µg/mL PMBN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	5 ± 6 (@25 µM)	30 ± 16 (@25 µM)	2 ± 5 (@25 µM)	9 ± 7 (@25 µM)	–4 ± 3 (@25 µM)	6 ± 1 (@25µM)	19 ± 2 (@25 µM)	5.3 ± 0.6
3	2 ± 11 (@50 µM)	33 ± 3 (@50 µM)	6 ± 7 (@50 µM)	3 ± 4 (@50 µM)	–22 ± 19 (@25 µM)	7 ± 5 (@50 µM)	–3 ± 5 (@50 µM)	5.5 ± 0.3
8	n.d.	9 ± 13 (@50 µM)	n.d.	n.d.	n.d.	3 ± 11 (@50 µM)	n.d.	n.d.
9	n.d.	14 ± 10 (@50 µM)	n.d.	n.d.	n.d.	21 ± 1 (@50 µM)	n.d.	1.9 ± 0.4
10	n.d.	5 ± 8 (@50 µM)	n.d.	n.d.	n.d.	8 ± 4 (@50 µM)	n.d.	n.d.
11	1 ± 9 (@100 µM)	46 ± 12 (@100 µM)	–29 ± 3 (@100 µM)	–2 ± 1 (@100 µM)	–36 ± 4 (@100 µM)	11 ± 1 (@100 µM)	n.d.	5.1 ± 0.5
12	1 ± 9 (@50 µM)	41 ± 17 (@50 µM)	14 ± 3 (@50 µM)	1 ± 7 (@100 µM)	–29 ± 24 (@50 µM)	–2 ± 12 (@50 µM)	n.d.	n.d.
13	–1 ± 1 (@100 µM)	38 ± 14 (@100 µM)	n.d.	n.d.	n.d.	10 ± 0 (@100 µM)	n.d.	n.d.
14	–2 ± 0 (@100 µM)	42 ± 15 (@100 µM)	n.d.	n.d.	n.d.	12 ± 10 (@100 µM)	n.d.	n.d.
15	2 ± 2 (@100 µM)	58 ± 17 (@100 µM)	n.d.	n.d.	n.d.	52 ± 15 (@100 µM)	n.d.	n.d.
16	13 ± 0 (@100 µM)	57 ± 2 (@100 µM)	4 ± 8 (@100 µM)	3 ± 3 (@100 µM)	18 ± 1 (@100 µM)	6 ± 3 (@100 µM)	19 ± 12 (@50 µM)	n.d.
17	13 ± 2 (@100 µM)	55 ± 14 (@100 µM)	65 ± 1 (@100 µM)	20 ± 4 (@100 µM)	16 ± 10 (@100 µM)	14 ± 3 (@50 µM)	23 ± 5 (@100 µM)	n.d.
21	8 ± 1 (@100 µM)	21 ± 2 (@100 µM)	n.d.	n.d.	n.d.	17 ± 2 (@100 µM)	n.d.	n.d.
22	2 ± 3 (@100 µM)	–3 ± 1 (@100 µM)	n.d.	n.d.	n.d.	10 ±12 (@100 µM)	n.d.	n.d.

23	15 ± 2 (@100 µM)	34 ± 10 (@100 µM)	n.d.	n.d.	n.d.	16 ± 4 (@100 µM)	n.d.	n.d.
24	10 ± 13 (@100 µM)	17 ± 1 (@100 µM)	1 ± 2 (@100 µM)	14 ± 1 (@100 µM)	–3 ± 16 (@100 µM)	5 ± 3 (@100 µM)	n.d.	n.d.
25	18 ± 11 (@100 µM)	32 ± 1 (@100 µM)	11 ± 6 (@100 µM)	18 ± 3 (@100 µM)	30 ± 7 (@100 µM)	11 ± 1 (@100 µM)	n.d.	n.d.
26	22 ± 4 (@100 µM)	10 ± 5 (@100 µM)	6 ± 1 (@100 µM)	15 ± 3 (@100 µM	–20 ± 19 (@100 µM)	–2 ± 5 (@100 µM)	17 ± 17 (@100 µM)	n.d.

* PMBN: polymyxin B nonapeptide, n.d.: not determined.

Table S4 Inhibitory activities against a panel of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, from at least two independent determinations.

	Percentage Inhibition @ 100 µM	
Compound	***P. aeruginosa***	***A. baumannii***
17	11 ± 7	31 ± 17
26	3 ± 9	12 ± 9

2.) Synthetic Procedures

2.1 General Procedures

General Procedure A: Amide Couplings[1]

Respective *N*-Boc protected amino acid (1.0 eq.), amine as either 2-aminothiazole derivative **6** or **7**, or 4-aminothiazole derivative **20** (1.0 eq.) and HBTU (1.2 eq.) were stirred under N_2 flow in DMF (1.5 mL) for 5 min, followed by an addition of triethylamine (3.0 eq.). The reaction mixture was stirred for 18 h. The mixture was quenched with saturated aq. $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with aq. 2 M HCl and brine. The organic layer was then dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was absorbed onto ISOLUTE® HM-N and purified by FCC with an alternating gradient of 0–80% EtOAc in cyclohexane to afford the title compounds **8–12** and **21–23**. Compounds **8–12** were further purified by prep. HPLC eluting with a gradient of 5–100% MeCN with 0.05% FA in H_2O with 0.05% FA. Collected fractions were lyophilised to afford the respective title compounds.

General Procedure B: Boc-deprotections

Boc-protected intermediates **8–12** and **21–23** was dissolved in DCM and TFA was added dropwise, in ratio of 5:1 or 10:1. The reaction mixture was stirred at room temperature for 30 min and then evaporated to dryness under reduced pressure. The obtained crude product was purified by prep. HPLC eluting with a gradient of 5–100% MeCN with 0.1% TFA in H_2O with 0.1% TFA. Collected fractions were lyophilised to afford the respective title compounds **13–17** and **24–26** as TFA salts. The TFA salt form of the compounds was measured using 4-(trifluoromethyl)phenylacetonitrile as an internal spike control.

General Abbreviations: aq. = aqueous, DCM = dichloromethane, DMF = dimethylformamide, eq. = equivalent, EtOAc = ethyl acetate, EtOH = ethanol, FA = formic acid, HCl = hydrochloric acid, HBTU = (2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate, MeCN = acetonitrile, $NaHCO_3$ = sodium bicarbonate, NaOH = sodium hydroxide, Na_2SO_4 = sodium sulfate, prep. = preparative, TEA = triethylamine and TFA = trifluoroacetic acid.

2.2 Reference Compounds

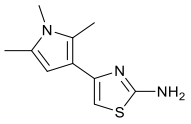
The reference compound **1** originated from previous publication within the consortium. Reference compounds **2** and **3** were commercially available compounds that were kindly provided by BASF. The purity of compounds was confirmed with LCMS.

Table S5 Reference compounds.

Compound	MW	LCMS $[M+H]^+$	UV-purity	Origin
1	354.44	355.1	>99%	Ref. 2
2	329.42	330.2	92%	Enamine Z26672805 (CAS 2094230-26-7)
3	345.48	346.1	~80%	Enamine Z26672672 (CAS 2391905-54-5)

2.3 Right-Hand Side Synthesis (Compounds 4–17 from Scheme 1)

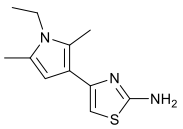
4-(1,2,5-Trimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine (6)[3]



To a stirred solution of 2-chloro-1-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)ethan-1-one **4** (0.500 g, 2.702 mmol) in EtOH (5 mL), thiourea (0.206 g, 2.702 mmol) was added. The mixture was refluxed for 16 h with an addition of thiourea (0.2 eq.) after 7 h. The reaction was quenched with water (35 mL) and washed with DCM (2 x 25 mL). The aqueous phase was concentrated *in vacuo*. The crude was dissolved in minimum amount of water (10 mL) and basified to pH 8 with aq. 2 M NaOH. The precipitate was filtered and vacuum dried to afford compound **6** as a brown sticky oil, (0.278 g, 50%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 6.76 (s, 2H), 6.14 (s, 1H), 5.92 (s, 1H), 3.34 (s, 3H), 2.40 (s, 3H), 2.12 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 167.1, 148.2, 126.4, 124.7, 114.6, 104.7, 96.4, 29.8, 12.2, 11.4. m/z (ESI+) 208.1 $[M + H]^+$.

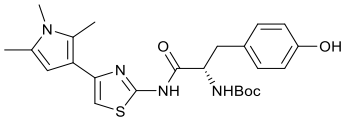
4-(1-Ethyl-2,5-dimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine (7)[3]



To a stirred solution of 2-chloro-1-(1-ethyl-2,5-dimethyl-1*H*-pyrrol-3-yl)ethan-1-one **5** (0.500 g, 2.504 mmol) in EtOH (5 mL), thiourea (0.191 mg, 2.504 mmol) was added. The mixture was refluxed for 16 h with an addition of thiourea (0.2 eq.) after 7 h. The reaction was quenched with water (35 mL) and washed with DCM (2 x 25 mL). The aqueous phase was concentrated *in vacuo*. The crude was dissolved in minimum amount of water (10 mL) and basified to pH 8 with aq. 2 M NaOH. The precipitate was filtered and vacuum dried to afford compound **7** as a brown sticky oil, (0.135 g, 24%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 6.77 (s, 2H), 6.16 (s, 1H), 5.92 (d, 1H, *J*=0.9), 3.79 (q, 2H, *J*=7.2 Hz), 2.43 (s, 3H), 2.16 (s, 3H), 1.15 (t, 3H, *J*=7.2 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 167.1, 148.2, 125.6, 123.8, 114.9, 105.2, 96.4, 37.4, 15.9, 11.9, 11.1. m/z (ESI+) 222.1 $[M + H]^+$.

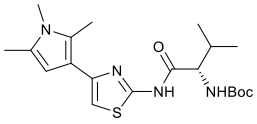
***Tert*-butyl (*S*)-(3-(4-hydroxyphenyl)-1-oxo-1-((4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)amino)propan-2-yl)carbamate (8)**



Using general procedure A with heating at 80 °C, *N*-Boc-L-tyrosine (0.060 g, 0.213 mmol) was coupled with 4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine **6** (0.044 g, 0.213 mmol), HBTU (0.097 g, 0.256 mmol) and TEA (9 µL, 0.640 mmol) in DMF (1.5 mL) to afford FCC purified compound **8** as a brown sticky oil (0.035 g, 35%). Further prep. HPLC purification (0.015 g) afforded compound **8** as a beige powder, (0.005 g).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.98 (br d, 2H, *J* = 7.6 Hz), 6.70 (br d, 2H, *J* = 7.6 Hz), 6.63 (s, 1H), 6.11 (s, 1H), 5.07 (br d, 1H, *J* = 5.2 Hz), 4.4-4.6 (m, 1H), 3.41 (s, 3H), 3.05-3.12 (m, 1H), 2.95-3.03 (m, 1H), 2.39 (s, 3H), 2.22 (s, 3H), 1.41 (br s, 9H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.1, 163.0, 156.5, 155.4, 147.2, 130.2, 127.7, 126.9, 125.0, 114.8, 113.9, 104.8, 103.3, 78.2, 56.2, 36.2, 29.9, 28.1, 12.2, 11.4. HRMS (ESI+) calcd. for $C_{24}H_{30}N_4O_4S$ $[M + H]^+$: 471.20606, found: 471.20422.

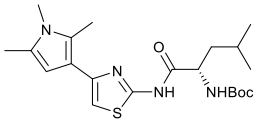
***Tert*-butyl (*S*)-(3-methyl-1-oxo-1-((4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)amino)butan-2-yl)carbamate (9)**



Using general procedure A, *N*-Boc-L-valine (0.050 g, 0.230 mmol) was coupled with 4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine **6** (0.048 g, 0.230 mmol), HBTU (0.105 g, 0.276 mmol) and TEA (10 µL, 0.690 mmol) in DMF (1.5 mL) to afford FCC purified compound **9** as a brown sticky oil (0.037 g, 40%). Further prep. HPLC purification (0.017 g) afforded compound **9** as a beige powder, (0.004 g).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.03 (s, 1H), 7.06 (br d, 1H, *J*=8.4 Hz), 6.77 (s, 1H), 6.01 (s, 1H), 4.05 (br t, 1H, *J*=7.1 Hz), 3.36 (br s, 3H), 2.44 (s, 3H), 2.15 (s, 3H), 1.9-2.0 (m, 1H), 1.37 (s, 9H), 0.87 (br t, 6H, *J*=7.1 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 170.6, 155.3, 147.0, 126.6, 124.8, 113.6, 104.5, 103.0, 77.9, 59.5, 29.8, 29.6, 27.9, 18.8, 18.2, 11.9, 11.1. HRMS (ESI+) calcd. for $C_{20}H_{30}N_4O_3S$ [M + H]$^+$: 407.21114, found: 407.20929.

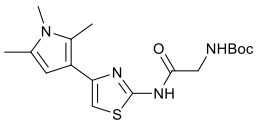
***Tert*-butyl (*S*)-(4-methyl-1-oxo-1-((4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)amino)pentan-2-yl)carbamate (10)**



Using general procedure A, *N*-Boc-L-leucine (0.050 g, 0.216 mmol) was coupled with 4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine **6** (0.045 g, 0.216 mmol), HBTU (0.099 g, 0.259 mmol) and TEA (9 µL, 0.649 mmol) in DMF (1.5 mL) to afford FCC purified compound **10** as a brown sticky oil (0.055 g, 60%). Further prep. HPLC purification (0.025 g) afforded compound **10** as a light beige powder, (0.007 g).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.06 (s, 1H), 7.16 (br d, 1H, *J*=7.6 Hz), 6.77 (s, 1H), 6.02 (s, 1H), 4.2-4.3 (m, 1H), 2.44 (s, 3H), 2.15 (s, 3H), 1.6-1.7 (m, 1H), 1.5-1.6 (m, 1H), 1.40-1.45 (m, 1H), 1.36 (s, 9H), 0.88 (br t, 6H, *J*=5.4 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.8, 156.4, 155.4, 147.1, 126.8, 125.0, 113.8, 104.7, 103.2, 78.1, 52.6, 29.8, 28.1, 24.3, 22.9, 21.2, 12.1, 11.3. HRMS (ESI+) calcd. for $C_{21}H_{32}N_4O_3S$ [M + H]$^+$: 421.22679, found: 421.22486.

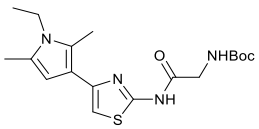
***Tert*-butyl (2-oxo-2-((4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)amino)ethyl)carbamate (11)**



Using general procedure A, *N*-Boc-glycine (0.040 g, 0.228 mmol) was coupled with 4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine **6** (0.047 g, 0.228 mmol), HBTU (0.104 g, 0.274 mmol) and TEA (10 µL, 0.685 mmol) in DMF (1.5 mL) to afford FCC purified compound **11** as a brown sticky oil (0.038 g, 46%). Further prep. HPLC purification (0.018 g) afforded compound **11** as a beige-rosa powder, (0.005 g).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.01 (br s, 1H), 7.14 (t, 1H, J=6.1 Hz), 6.78 (s, 1H), 6.02 (s, 1H), 3.83 (d, 2H, J=6.1 Hz), 3.37 (s, 3H), 2.44 (s, 3H), 2.15 (s, 3H), 1.40 (s, 9H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 168.5, 156.6, 156.1, 147.4, 127.1, 125.2, 114.1, 105.0, 103.3, 78.4, 43.1, 30.1, 28.4, 12.4, 11.6. HRMS (ESI+) calcd. for $C_{17}H_{24}N_4O_3S$ [M + H]$^+$: 365.16419, found: 365.16255.

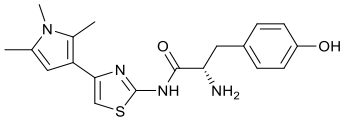
***Tert*-butyl (2-((4-(1-ethyl-2,5-dimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)amino)-2-oxoethyl)carbamate (12)**



Using general procedure A, *N*-Boc-glycine (0.035 g, 0.200 mmol) was coupled with 4-(1-ethyl-2,5-dimethyl-1*H*-pyrrol-3-yl)thiazol-2-amine **7** (0.044 g, 0.200 mmol), HBTU (0.091 g, 0.240 mmol) and TEA (8 µL, 0.599 mmol) in DMF (1.5 mL) to afford FCC purified compound **12** as a brown sticky oil (0.062 g, 82%). Further prep. HPLC purification (0.022 g) afforded compound **12** as a white powder, (0.005 g).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.00 (br s, 1H), 7.14 (br t, 1H, J=6.0 Hz), 6.78 (s, 1H), 6.01 (s, 1H), 3.8-3.9 (m, 4H), 2.46 (br s, 3H), 2.15-2.20 (m, 3H), 1.3-1.4 (m, 9H), 1.16 (br t, 3H, J=7.1 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 168.2, 156.3, 155.8, 147.1, 126.0, 124.1, 114.0, 105.1, 103.1, 78.1, 42.8, 37.4, 28.1, 15.8, 11.8, 11.0. HRMS (ESI+) calcd. for $C_{18}H_{26}N_4O_3S$ [M + H]$^+$: 379.17984, found: 379.17789.

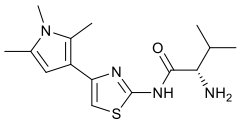
(*S*)-2-Amino-3-(4-hydroxyphenyl)-*N*-(4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)propanamide (13)



Using general procedure B, compound **8** (0.020 g, 0.186 mmol) was Boc-deprotected in DCM (1 mL) with TFA (0.2 mL) to afford prep. HPLC purified compound **13** as a purple powder one TFA salt form, (0.003 g, 17%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.54 (br s, 1H), 9.3-9.5 (m, 1H), 8.2-8.4 (m, 4H), 7.04 (d, 2H, J=8.4 Hz), 6.88 (s, 1H), 6.71 (d, 2H, J=8.4 Hz), 6.02 (s, 1H), 4.16-4.22 (m, 1H), 3.37 (s, 3H), 3.08-3.13 (m, 1H), 2.9-3.0 (m, 1H), 2.54 (s, 3H), 2.43 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 166.9, 158.1, 157.8, 156.5, 154.4, 130.3, 126.9, 125.0, 124.2, 115.3, 104.6, 103.9, 53.9, 35.9, 29.8, 12.0, 11.2. HRMS (ESI+) calcd. for $C_{19}H_{22}N_4O_2S$ $[M + H]^+$: 371.15363, found: 371.15204.

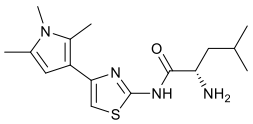
(*S*)-2-Amino-3-methyl-*N*-(4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)butanamide (14)



Using general procedure B, compound **9** (0.020 g, 0.049 mmol) was Boc-deprotected in DCM (1 mL) with TFA (0.2 mL) to afford prep. HPLC purified compound **14** as a light pink powder one TFA salt form, (0.014 g, 68%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.60 (br s, 1H), 8.34 (br s, 4H), 6.90 (s, 1H), 6.03 (m, 1H), 3.86-3.89 (m, 2H), 3.37 (s, 3H), 2.44 (s, 3H), 2.2-2.3 (m, 1H), 2.15 (s, 3H), 0.97 (dd, 6H, J=6.9, 10.8 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 158.3, 158.0, 147.7, 127.1, 125.3, 113.6, 104.8, 104.1, 57.6, 29.9, 18.5, 17.6, 12.2, 11.4. HRMS (ESI+) calcd. for $C_{15}H_{22}N_4OS$ $[M + H]^+$: 307.15871, found: 307.15727.

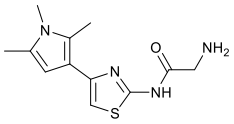
(*S*)-2-Amino-4-methyl-*N*-(4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)pentanamide (15)



Using general procedure B, compound **10** (0.030 g, 0.071 mmol) was Boc-deprotected in DCM (1.5 mL) with TFA (0.3 mL) to afford prep. HPLC purified compound **15** as a rosa powder one TFA salt form, (0.013 g, 41%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.68 (br s, 1H), 8.35 (br s, 4H), 6.90 (s, 1H), 6.04 (s, 1H), 4.07-4.10 (m, 1H), 3.38 (s, 3H), 2.45 (s, 3H), 2.16 (s, 3H), 1.6-1.7 (m, 3H), 0.94 (br d, 6H, J=4.0 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 158.2, 158.0, 127.1, 125.3, 115.3, 104.8, 104.1, 51.1, 30.0, 23.7, 22.7, 21.6, 12.2, 11.4. HRMS (ESI+) calcd. for $C_{16}H_{24}N_4OS$ [M + H]$^+$: 321.17436, found: 321.17288.

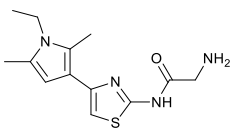
2-Amino-*N*-(4-(1,2,5-trimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)acetamide (16)



Using general procedure B, compound **11** (0.020 g, 0.055 mmol) was Boc-deprotected in DCM (1 mL) with TFA (0.2 mL) to afford prep. HPLC purified compound **16** as a rosa powder one TFA salt form, (0.008 g, 39%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.46 (br s, 1H), 8.18 (br s, 4H), 6.88 (s, 1H), 6.02 (d, 1H, J=0.8 Hz), 3.89 (br d, 2H, J=5.5 Hz), 3.37 (s, 3H), 2.44 (s, 3H), 2.15 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 157.9, 127.1, 125.2, 113.7, 104.8, 103.8, 40.7, 30.0, 12.2, 11.4. HRMS (ESI+) calcd. for $C_{12}H_{16}N_4OS$ [M + H]$^+$: 265.11176, found: 265.11059.

2-Amino-*N*-(4-(1-ethyl-2,5-dimethyl-1*H*-pyrrol-3-yl)thiazol-2-yl)acetamide (17)

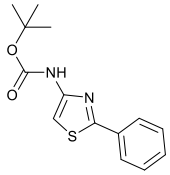


Using general procedure B, compound **12** (0.040 g, 0.106 mmol) was Boc-deprotected in DCM (2 mL) with TFA (0.4 mL) to afford prep. HPLC purified compound **17** as a pink powder one TFA salt form, (0.006 g, 14%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 12.44 (br s, 1H), 8.19 (br s, 4H), 6.87 (s, 1H), 6.02 (s, 1H), 3.87-3.91 (m, 2H), 3.82 (q, 3H, J=7.1 Hz), 2.46 (s, 3H), 2.18 (s, 3H), 1.17 (br t, 4H, J=7.1 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz,) δ 158.2, 157.9, 126.3, 124.4, 113.7, 105.2, 103.8, 40.7, 37.6, 15.9, 11.9, 11.1. HRMS (ESI+) calcd. for $C_{13}H_{18}N_4OS$ [M + H]$^+$: 279.12741, found: 279.12604.

2.4 Left-Hand Side Synthesis (Compounds 18-26 from Scheme 2)

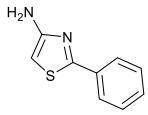
Tert-butyl (2-phenylthiazol-4-yl)carbamate (19)[4]



To a suspension of 2-phenylthiazole-4-carboxylic acid **18** (0.500 g, 2.439 mmol) in *t*BuOH (6.8 mL), DPPA (0.63 mL, 2.926 mmol) and TEA (0.41 mL, 2.926 mmol) were added. The mixture was stirred in a pressure vial at 80 °C for 16 h. The reaction mixture was cooled down to room temperature and concentrated to dryness. The crude was dissolved in EtOAc (10 mL), washed with water (5 mL) and saturated aq. $NaHCO_3$ (5 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The crude product was absorbed onto ISOLUTE® HM-N and purified by FCC with a gradient of 0–20% EtOAc in cyclohexane to afford the compound **19** as an off-white powder, (0.345 g, 51%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.86-7.92 (m, 2H), 7.53 (br s, 1H), 7.4-7.5 (m, 3H), 7.24 (br s, 1H), 1.54 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 165.5, 152.6, 148.4, 133.3, 130.4, 129.2, 126.3, 98.5, 28.5, 27.2. m/z (ESI+) 277.1 $[M + H]^+$.

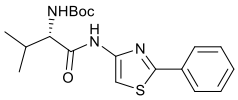
2-Phenylthiazol-4-amine (20)[4]



To a solution of *tert*-butyl (2-phenylthiazol-4-yl)carbamate **19** (0.300 g, 1.086 mmol) in dioxane (2.4 mL), 4M HCl/dioxane solution (3.9 mL) was added. The mixture was stirred at room temperature for 16 h. Organic phase was evaporated leaving the acidic aqueous layer that was extracted with Et_2O (2 x 10 mL). Combined organic layers were further washed with aq. 1 M HCl solution (15 mL). The combined aqueous layers were basified with 10% NaOH (aq.) and extracted with DCM (2 x 20 mL). The organic layers were then combined, dried over anhydrous Na_2SO_4, filtered and evaporated to dryness to afford compound **20** as an orange-brown oil, (0.181 g, 95%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.80-7.83 (m, 2H), 7.4-7.5 (m, 3H), 5.92 (s, 1H), 5.45 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 163.2, 158.8, 133.2, 129.2, 128.8, 124.9, 87.1. m/z (ESI+) 177.1 $[M + H]^+$.

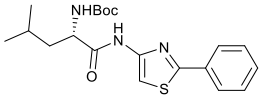
***Tert*-butyl (*S*)-(3-methyl-1-oxo-1-((2-phenylthiazol-4-yl)amino)butan-2-yl)carbamate (21)**



Using general procedure A, *N*-Boc-L-valine (0.045 g, 0.207 mmol) was coupled with 2-phenylthiazol-4-amine **20** (0.037 g, 0.207 mmol), HBTU (0.095 g, 0.249 mmol) and TEA (9 µL, 0.621 mmol) in DMF (1.5 mL) with addition of *N*-Boc-L-valine, HBTU and TEA (1.0 eq.) after 16 h continued in total reaction time of 48 h at room temperature to afford FCC purified compound **21** as a yellow oil (0.011 g, 15%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.01 (br s, 1H), 7.89-7.93 (m, 2H), 7.69 (s, 1H), 7.5-7.5 (m, 3H), 5.0-5.1 (m, 1H), 4.1-4.2 (m, 1H), 2.3-2.4 (m, 1H), 1.48 (s, 9H), 1.05 (d, 3H, *J*=6.7 Hz), 0.98 (d, 3H, *J*=6.7 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 169.8, 165.7, 156.2, 147.3, 133.3, 130.5, 129.3, 126.4, 102.3, 80.7, 60.6, 28.5, 27.1, 19.6. HRMS (ESI+) calcd. for $C_{19}H_{25}N_3O_3S$ $[M + H]^+$: 376.16894, found: 376.16869.

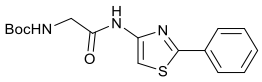
***Tert*-butyl (*S*)-(4-methyl-1-oxo-1-((2-phenylthiazol-4-yl)amino)pentan-2-yl)carbamate (22)**



Using general procedure A, *N*-Boc-L-leucine (0.045 g, 0.195 mmol) was coupled with 2-phenylthiazol-4-amine **20** (0.034 g, 0.195 mmol), HBTU (0.089 g, 0.233 mmol) and TEA (8 µL, 0.584 mmol) in DMF (1.5 mL) with addition of *N*-Boc-L-leucine, HBTU and TEA (1.0 eq.) after 16 h, continued in total reaction time of 48 h at room temperature to afford FCC purified compound **22** as a yellow oil (0.017 g, 22%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.02 (br s, 1H), 7.89-7.93 (m, 2H), 7.65 (s, 1H), 7.4-7.5 (m, 3H), 4.8-5.0 (m, 1H), 4.3-4.4 (m, 1H), 1.7-1.9 (m, 2H), 1.55-1.62 (m, 1H), 1.48 (s, 9H), 0.97-1.01 (m, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 170.5, 165.3, 151.2, 146.9, 132.6, 130.4, 129.1, 126.2, 101.9, 80.7, 53.5, 41.2, 29.7, 28.3, 24.8, 23.0. HRMS (ESI+) calcd. for $C_{20}H_{27}N_3O_3S$ $[M + H]^+$: 390.18459, found: 390.18440.

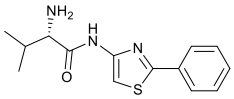
***Tert*-butyl (2-oxo-2-((2-phenylthiazol-4-yl)amino)ethyl)carbamate (23)**



Using general procedure A, *N*-Boc-glycine (0.035 g, 0.200 mmol) was coupled with 2-phenylthiazol-4-amine **20** (0.035 g, 0.200 mmol), HBTU (0.091 g, 0.240 mmol) and TEA (8 µL, 0.599 mmol) in DMF (1.5 mL) to afford FCC purified compound **23** as a yellow oil (0.024 g, 35%).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.94 (br s, 1H), 7.87-7.92 (m, 2H), 7.66 (s, 1H), 7.4-7.5 (m, 3H), 5.22 (br s, 1H), 4.03 (br s, 2H), 1.50 (s, 9H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 167.0, 165.1, 155.8, 146.5, 132.5, 130.1, 128.8, 126.0, 101.9, 80.5, 44.5, 28.0. HRMS (ESI+) calcd. for $C_{16}H_{19}N_3O_3S$ $[M + H]^+$: 334.12199, found: 334.12169.

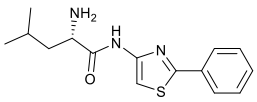
(*S*)-2-Amino-3-methyl-*N*-(2-phenylthiazol-4-yl)butanamide (24)



Using general procedure B, compound **21** (0.008 g, 0.021 mmol) was Boc-deprotected in DCM (0.9 mL) with TFA (0.1 mL) to afford prep. HPLC purified compound **24** as a white powder one TFA salt form, (0.005 g, 58%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.64 (br s, 1H), 8.2-8.3 (m, 3H), 7.90-7.94 (m, 2H), 7.74 (s, 1H), 7.5-7.6 (m, 3H), 3.8-3.9 (m, 1H), 3.7-3.8 (m, 1H), 2.1-2.2 (m, 1H), 0.99 (t, 6H, J=7.2 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.2, 157.6, 147.6, 131.0, 130.1, 129.8, 129.5, 126.2, 103.5, 58.1, 30.4, 25.9, 18.8, 18.1. HRMS (ESI+) calcd. for $C_{14}H_{17}N_3OS$ $[M + H]^+$: 276.11651, found: 276.11639.

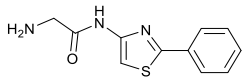
(*S*)-2-Amino-4-methyl-*N*-(2-phenylthiazol-4-yl)pentanamide (25)



Using general procedure B, compound **22** (0.014 g, 0.036 mmol) was Boc-deprotected in DCM (1.1 mL) with TFA (0.11 mL) to afford prep. HPLC purified compound **25** as an off-white powder one TFA salt form, (0.006 g, 40%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.74 (br s, 1H), 8.2-8.3 (m, 3H), 7.90-7.94 (m, 2H), 7.73 (s, 1H), 7.51-7.54 (m, 3H), 4.0-4.1 (m, 1H), 1.6-1.7 (m, 3H), 0.94 (t, 6H, J=6.3 Hz). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 168.0, 165.1, 147.7, 132.9, 130.9, 129.7, 126.1, 103.4, 51.6, 24.1, 23.0, 22.1. HRMS (ESI+) calcd. for $C_{15}H_{19}N_3OS$ $[M + H]^+$: 290.13216, found: 290.13187.

2-Amino-*N*-(2-phenylthiazol-4-yl)acetamide (26)



Using general procedure B, compound **23** (0.018 g, 0.054 mmol) was Boc-deprotected in DCM (1.8 mL) with TFA (0.2 mL) to afford prep. HPLC purified compound **26** as a beige powder one TFA salt form, (0.009 g, 45%).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 11.54 (s, 1H), 8.15 (br s, 3H), 7.89-7.93 (m, 2H), 7.68 (s, 1H), 7.50-7.55 (m, 3H), 3.83 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 165.2, 165.1, 147.9, 133.0, 131.0, 129.9, 126.2, 102.9, 41.2. HRMS (ESI+) calcd. for $C_{11}H_{11}N_3OS$ $[M + H]^+$: 234.06956, found: 234.06944.

3.) References

1) B. Liu, G. Liu, L. Nelson, J. Patel, H. Sham, Z. Xin, H. Zhao, US20050014794 A1, 2005.

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4) C. Bolea, WO2011/86163A1, ADDEX PHARMA S.A., 2011.